Response to Office Action of July 14, 2008 Attorney Docket: NOTAR-031US

Amendments to the Claims:

 (Currently Amended) Polysaccharide double-layer microcapsules constituted by an outer layer of chitosan and an inner layer of alginate wherein they are obtained:

- from solutions of alginate with initial concentrations ranging from 2 to 4% w/v comprising the further polymer hydroxypropylmethylcellulose at the initial concentration of 0.4% w/v:
- from solutions of chitosan with initial concentrations ranging from 0.1 to 0.5% $\mbox{w/v}$;
- from solutions of divalent ions with concentrations of 0.5% w/v, when the divalent ion functions as a gelification agent of the alginate to form single-layer capsules of alginate encapsulating at least one biologically active substance, and ranging from 10 to 15% w/v when the divalent ion has a stabilizing function of the double layer eapsules microcapsule's outer coating of chitosan;

wherein said at least one biologically active substance is lysozyme:

for use as carriers for the oral administration of said biologically active substances.

- (Cancelled)
- 3. (Currently Amended) Polysaccharide double-layer microcapsules as claimed in claim 1, wherein the initial concentration of alginate is 4% w/v, the initial concentration of chitosan is 0.1% w/v and the divalent ion with stabilizing function on the double layer microcapsules microcapsule's outer coating of chitosan has an initial concentration of 15% w/v.
- (Original) Polysaccharide double-layer microcapsules as claimed in claim 1, wherein the divalent ion is calcium.
- 5. (Currently Amended) Polysaccharide double-layer microcapsules as claimed in claim 1, wherein <u>said lysozyme</u> is an <u>adjuvant</u>, and <u>further comprising at least one</u> the biologically active <u>substance</u> <u>substances</u> are selected from <u>the group consisting of immunomodulants</u>, antigens, chemotherapeutics, cytokines and growth factors.
 - 6-28 (Cancelled)
- (Currently Amended) Polysaccharide double-layer microcapsules constituted by an outer layer of chitosan and an inner layer of alginate wherein they are obtained through:

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a) formation of single-layer capsules encapsulating at least one biologically active substance starting from solutions of alginate in concentrations ranging from 2 to 4% w/v comprising the further polymer hydroxypropylmethylcellulose at the initial concentration of 0.4% w/v, in which said substance is dispersed, by gelification with a solution of a divalent ion at a concentration of 0.5% w/v;

b) formation of the second <u>coating</u> layer of chitosan and stabilization of the double-layer microcapsule obtained by adding a solution of chitosan in concentrations ranging from 0.1 to 0.5% w/v and containing a divalent ion in concentrations ranging from 10 to 15% w/v in the solution containing the single-layer capsules of alginate encapsulating said substance obtained in a):

wherein said at least one biologically active substance is lysozyme;

for use as carriers for the oral administration of said biologically active substances.

- (Cancelled)
- 31. (Previously Presented) Polysaccharide double-layer microcapsules as claimed in claim 29, wherein the initial concentration of alginate is 4% w/v, the initial concentration of chitosan is 0.1% w/v and the divalent ion with stabilizing function on the double layer microcapsules has an initial concentration of 15% w/v.
- (Previously Presented) Polysaccharide double-layer microcapsules as claimed in claim 29, wherein the divalent ion is calcium.
- 33. (Currently Amended) Polysaccharide double-layer microcapsules as claimed in claim 29, wherein <u>said_lysozyme is an adjuvant, and further comprising at least one the biologically active <u>substance substances—are</u> chosen from <u>the group consisting of immunomodulants</u>, antigens, chemotherapeutics, cytokines and growth factors.</u>

34-36 (Cancelled)

37. (Withdrawn) A method for vaccinogenic or therapeutic treatment for the prophylaxis and therapy of infectious or non-infectious diseases by administering to a mammal, human or not human, in need a pharmaceutical composition comprising polysaccharide double-layer microcapsules constituted by an outer layer of chitosan and an inner layer of alginate obtained:

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> from solutions of alginate with initial concentrations ranging from 2 to 4% w/v comprising the further polymer hydroxypropylmethylcellulose at the initial concentration of 0.4% w/v.

- from solutions of chitosan with initial concentrations ranging from 0.1 to $0.5\,\%$ w/v;
- from solutions of divalent ions with concentrations of 0.5% w/v, when the divalent ion functions as a gelification agent of the alginate to form single-layer capsules of alginate encapsulating at least one biologically active substance, and ranging from 10 to 15% w/v when the divalent ion has a stabilizing function of the double layer capsules.
- (Cancelled)
- 39. (Withdrawn) The method as claimed in claim 37, wherein the initial concentration of alginate is 4% w/v, the initial concentration of chitosan is 0.1% w/v and the divalent ion with stabilizing function on the double layer microcapsules has an initial concentration of 15% w/v.
- (Withdrawn) The method as claimed in claim 37, wherein the divalent ion is calcium.
- 41. (Withdrawn) The method as claimed in claim 37, wherein the biologically active substances are chosen from immunomodulants, antigens, chemotherapeutics, cytokines and growth factors.
- (Withdrawn) The method as claimed in claim 37, wherein the biologically active substance is lysozyme.
- 43. (Withdrawn) The method as claimed in claim 37, wherein an adjuvant is associated with the biologically active substance to increase the biological response.
- (Withdrawn) The method as claimed in claim 43, wherein the adjuvant is lysozyme.
- 45. (Withdrawn) The method as claimed in claim 37, wherein the prophylaxis and therapy of infectious or non-infectious diseases is applied in the animal breeding or fish farming field.
- 46. (Withdrawn) The method as claimed in claim 37, wherein the composition of polysaccharide double-layer microcapsules are in formulations suitable for oral administration selected from solid forms consisting of powders, tablets, capsules, or liquid forms consisting of

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oily or aqueous solutions, both for multiple dosage and in single doses with excipients or diluents acceptable from the pharmaceutical and feeding purposes in the human and veterinary field.

- 47. (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules constituted by an outer layer of chitosan and an inner layer of alginate comprising the following phases:
 - a) formation of single-layer capsules encapsulating at least one biologically active substance starting from solutions of alginate in concentrations ranging from 2 to 4% w/v comprising the further polymer hydroxypropylmethylcellulose at the initial concentration of 0.4% w/v, in which said substance is dispersed, by gelification with a solution of a divalent ion at a concentration of 0.5% w/v:
 - b) formation of the second layer of chitosan and stabilization of the double-layer microcapsule obtained by adding a solution of chitosan in concentrations ranging from 0.1 to 0.5% w/v and containing a divalent ion in concentrations ranging from 10 to 15% w/v in the solution containing the single-layer capsules of alginate encapsulating at least one biologically active substance obtained in a).
- 48. (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules as claimed in claim 47, wherein added to phases a) and b) is the phase c) of dehydration, isolation and drying of the microcapsules obtained.
 - 49. (Cancelled)
- 50. (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules as claimed in claim 47, wherein the initial concentration of alginate is 4% w/v, the initial concentration of chitosan is 0.1% w/v and the divalent ion with stabilizing function on the double layer microcapsules has an initial concentration of 15% w/v.
- (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules as claimed in claim 47, wherein the divalent ion is calcium.
- 52. (Withdrawn) Process for the preparation of microcapsules as claimed in claim 47, wherein the biologically active substances are chosen from immunomodulants, antigens, chemotherapeutics, cytokines and growth factors.
- (Withdrawn) Process for the preparation of microcapsules as claimed in claim 47, wherein the biologically active substance is lysozyme.

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54. (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules as claimed in claim 47, wherein an adjuvant is associated with the biologically active substance to increase the biological response.

55. (Withdrawn) Process for preparation of polysaccharide double-layer microcapsules as claimed in claim 54, wherein the adjuvant is lysozyme.